Bioinformatics for biomedicine

Gene expression data, methods of analysis

Lecture 7, 2006-10-31

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Course design

1. What is bioinformatics? Basic databases and tools
2. Sequence searches: BLAST, FASTA
3. Multiple alignments, phylogenetic trees
4. Protein domains and 3D structure
5. Seminar: Sequence analysis of a favourite gene
6. More annotation, Gene Ontology and pathways
7. **Gene expression data, methods of analysis**
8. Seminar: Further analysis of a favourite gene

Task

- Locate protein in GO, Reactome, etc
- Wee1
- SREBP1
- Your own

Task: Wee1

- GO via
  - UniProt (WEE1_HUMAN)
    - Protein kinase; cell cycle; nucleus
  - Ensembl
    - Mitosis (code IEA: Inferred from Electronic Annotation)
- Reactome
  - Phosphorylated by Chk1, Plk1; inactivation
  - Phosphorylates cyclins B1, E1, E2, A

Task: SREBP1

- GO
  - via UniProt
    - ER membrane, nuclear envelope, nucleus
    - Transcription factor; lipid metabolism
  - Via Ensembl
    - Steroid metabolism (IEA)
- Reactome: nothing
- KEGG: Insulin signaling pathway
  - Downstream of PI3K, PIP3, PKC iota
  - Regulates metabolic enzymes PFK, PyK, GK

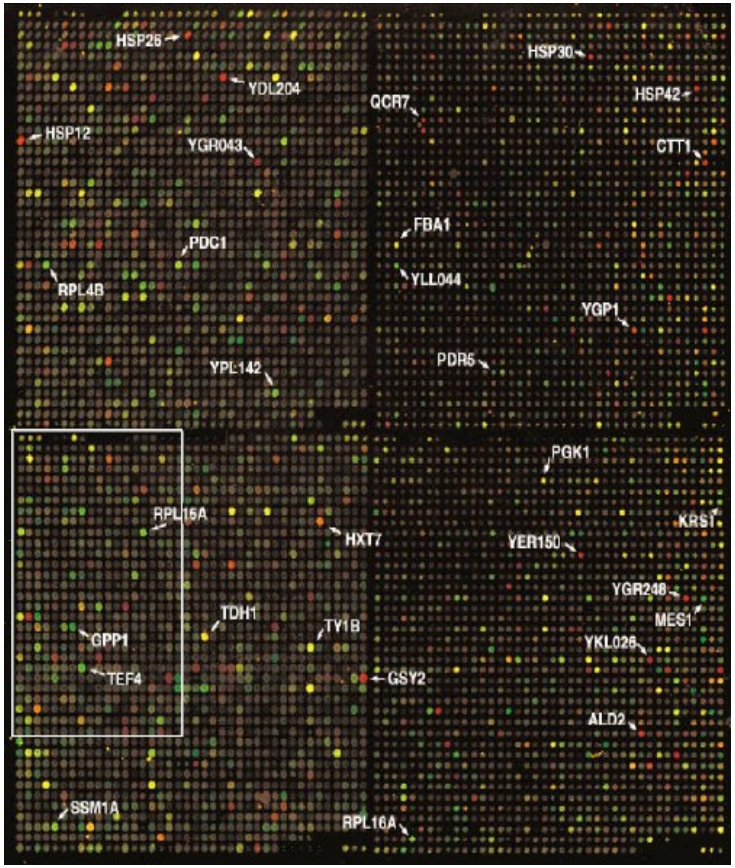
Gene activity; expression

- Gene expression = mRNA level
- Proxy for gene activity
  - Approximation (usually reasonable)
- Many technologies for measurement
  - Performance
    - Absolute leve/relative change
    - Accuracy
    - Throughput (arrays vs. samples)
    - Predefined gene set, or identification of new genes
  - Cost
    - Investment vs. running cost

Microarrays for gene expression

- Attach known oligonucleotides on a surface, spot by spot
- Hybridize with color-labelled sample
  - Relative: two samples with different color
  - Absolute: one single color
- Read off intensity of color in each spot
  - Convert to expression value for each gene
  - Relative change or absolute level

cDNA microarray
Pat Brown's lab, Stanford



Glass slide, cDNA spots
Two-color approach: relative change

Sample 1 (ref) is labelled green (Cy3)
Sample 2 (exp) is labelled red (Cy5)

Spot colors:
- Black: no mRNA; no change
- Green: exp mRNA downregulated
- Red: exp mRNA upregulated
- Yellow: ref and exp mRNA; no change

Yeast genome
DeRisi, Iyer & Brown
Science 278 (1997) 680-686

Why not absolute values?

- C = k * Ic
  - C : level of mRNA
  - k : proportionality constant
  - Ic : color intensity

- Absolute value C requires k
  - k different for each cDNA
  - Calibration needed

Why relative values?

- How to avoid calibration for k ?
- Experiment relative to reference
  - Measure up- or down-regulation

- $G = C_{exp}/C_{ref} = k*Ic_{exp}/k*Ic_{ref} = Ic_{exp}/Ic_{ref}$

- But: Equal amounts of total mRNA in the two samples (exp, ref)?

Data reduction issues

- Treatment from raw data to useful value
  - From spot shape/color to up/down regulation
  - Similar problem in many technologies
- Many steps
  - Depends on microarray technology
  - Define spot; shape, position
  - Measure color intensity; background?
  - Handle artifacts (damaged spots, etc)

Normalization

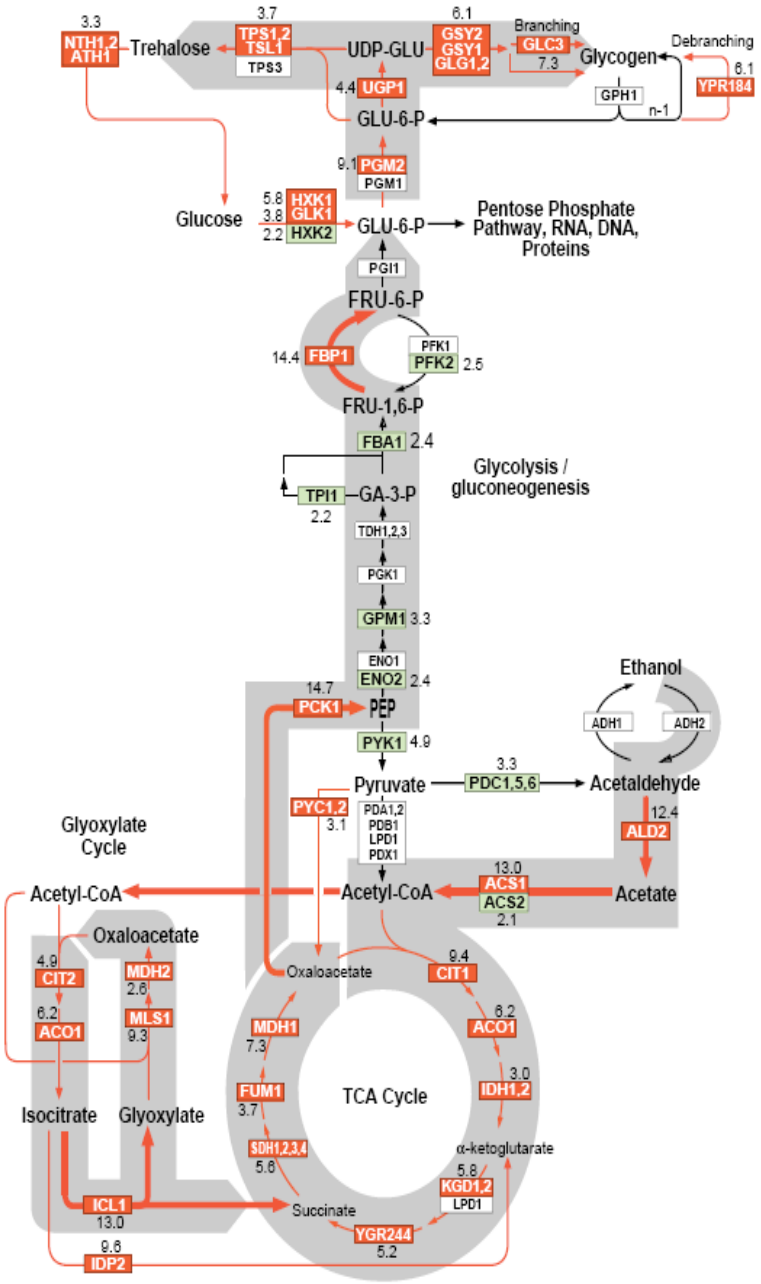
- Goal: Make data sets comparable
- Microarrays
  - Between colors in chip
  - Between chips in experiment
  - Between genes in different experiments
- Common approaches
  - Use constant gene(s); “house-keeping” genes
    - Ribosomal proteins
    - Fundamental metabolic enzymes
  - Danger: Based on assumptions!

Example analysis: Pathways

- Up/down regulation of genes
- Map onto known pathways
- Indicates changes in flows or signals
- Mechanistic information:
  - Verification of known data
  - Patterns
  - Interesting anomalies
- Assumes biological knowledge

Yeast: diauxic shift

- Green: anaerobic fermentation (glucose>ethanol)
- Red: aerobic respiration (ethanol>TCA cycle)
- Shows activation/ deactivation of pathways
- Behaviour of gene copies



DeRisi, Iyer & Brown
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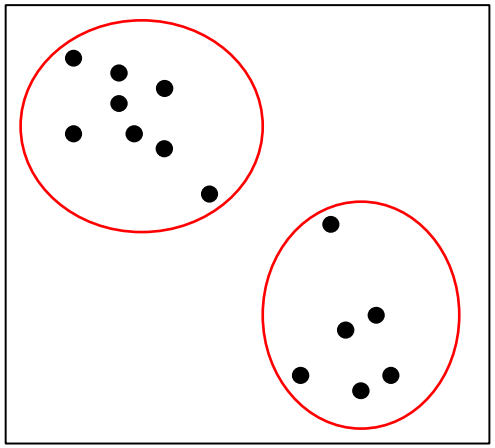
Clustering 1

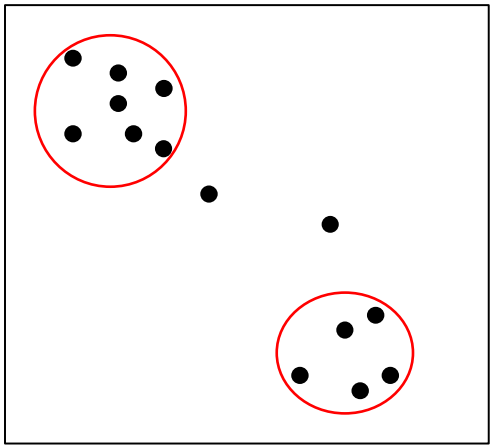
- Groups according to some property
- Computational
  - Measure of relationship; distance(i,j)
  - Many algorithms to form groups
- Powerful data analysis technique
  - Always some assumption on type of groups
  - No single optimal clustering method

Clustering 2

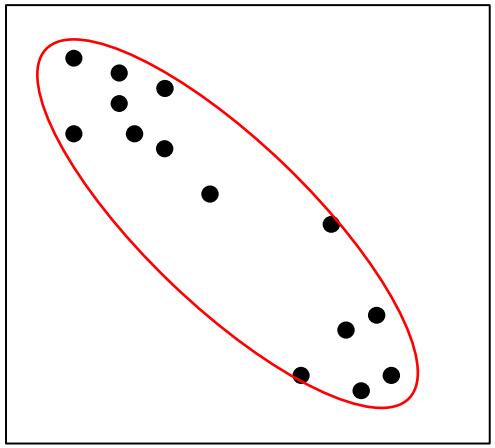
- A set of data points
- Two dimensions (x, y)
- How form groups?



All included
Roundness



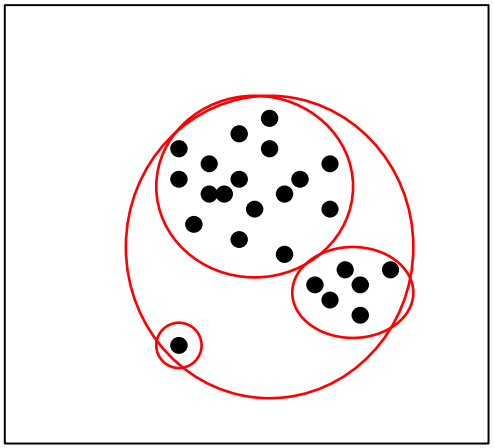
Tightness
Roundness



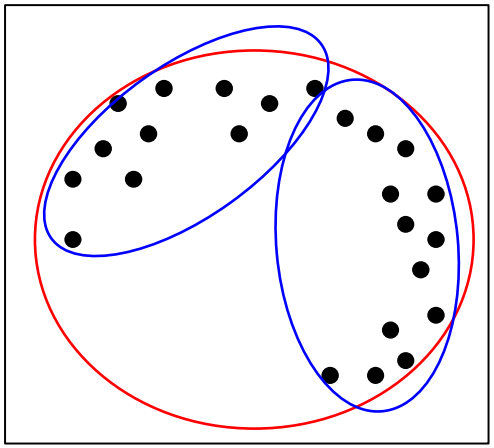
All included
Any shape

Clustering 3

- Depends on previous knowledge
  - What groups are expected?
  - How measure when assumptions violated?



Hierarchical



How notice problem?

Clustering 4

- Hierarchical clustering
  - Common in gene expression analysis
  - Useful, but not necessary
    - There is no intrinsic biological reason!
- Possible problems
  - Sensitive to minor errors
  - At what level “natural” clusters?
  - Hard to detect “strangely shaped” clusters

More than 2 dimensions

- More than one treatment
  - Ref + exp1 + exp2 + exp3 +…
- Time course experiment
  - Ref(t0), exp (t1), exp(t2), …
- Add other parameters
  - Anything of interest: pI, Mw,…
- Very common, and very useful

Scaling problem

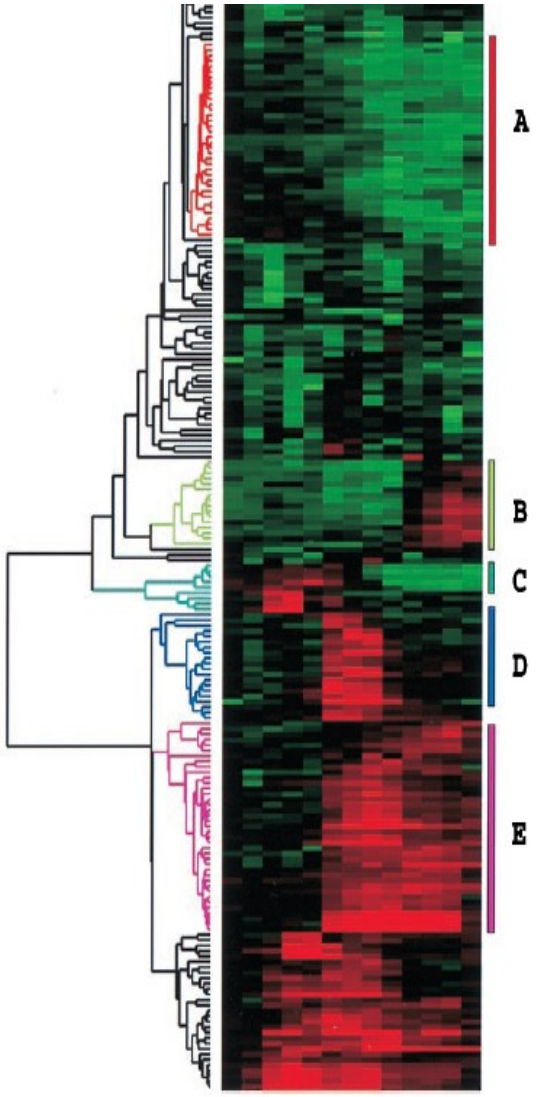
- How to compare different dimensions?
  - Expression value, pI, Mw,…
  - Distance functions require scaling
    - Making values comparable
- Possible approaches
  - Weights: Specific to each problem
  - Recalculation: Statistical
    - Same average
    - Same standard deviation

Clustering in many dimensions

- Can be generalized into N dimensions
- Each point a vector (s_i, s_j, …s_n)
- Distance between two points
  - Many possible functions; also called “metric”
    - Euclidean: $d = \mathrm{sqrt}(d_i^2 + d_j^2 + \ldots d_n^2)$
    - Manhattan: $d = |d_i| + |d_j| + \ldots |d_n|$
    - Correlation: similar “tendency” for values s

Example: Hierarchical clustering

- 12 values per gene
  - Time course, 0-24 hrs
- Clustering
  - Correlation coefficient metric
  - Hierarchical; dendrogram
  - No cutoff level
  - No test of significance



Eisen et al
PNAS 95 (1998) 14863-14868

Time course experiment 1

- Yeast diauxic shift
  - t=9: high glucose
  - t=19: low glucose
- Small sets of genes behaving similarly
- Biological analysis
  - F) Ribosomal proteins
  - E) Several uncharacterized

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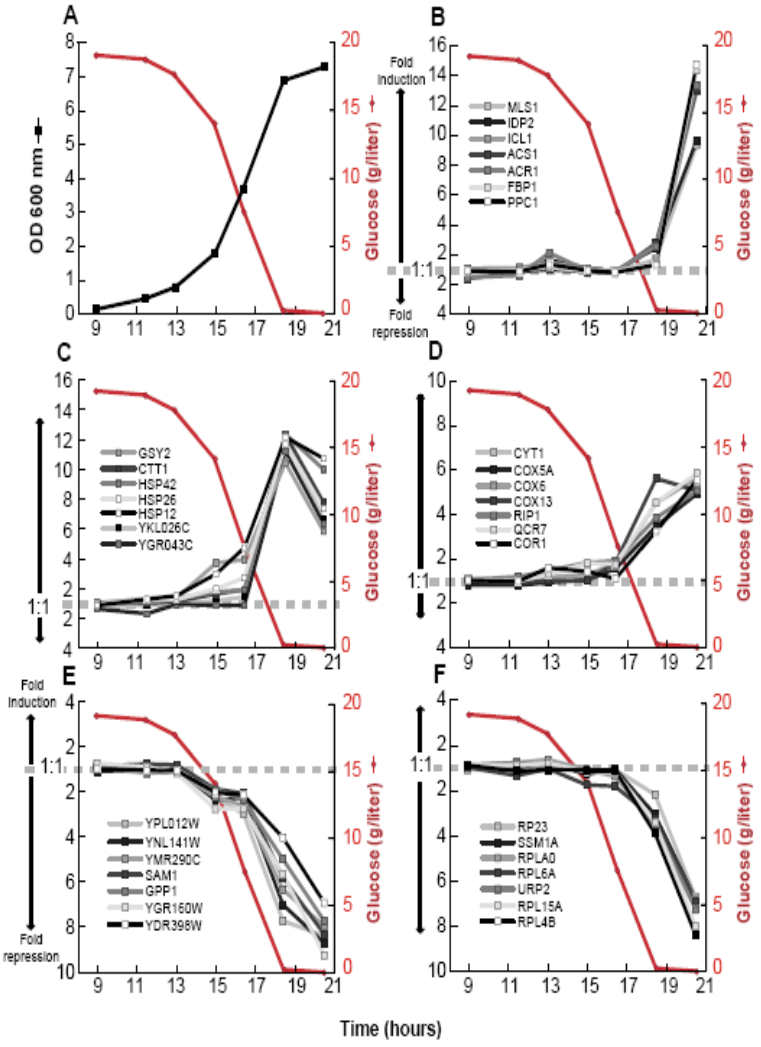
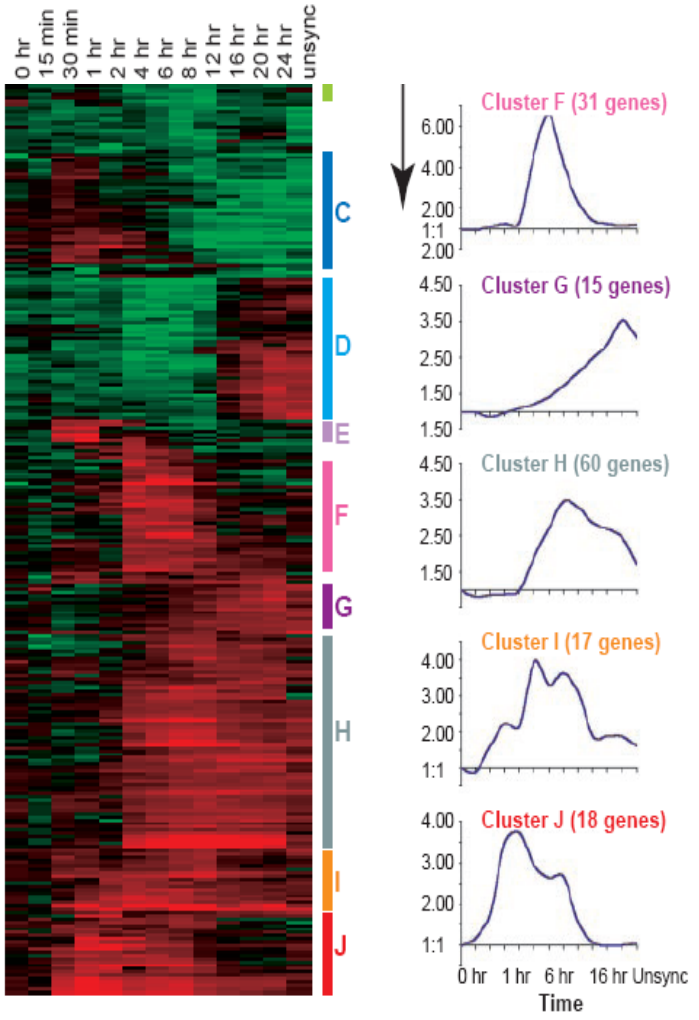


Time course experiment 2

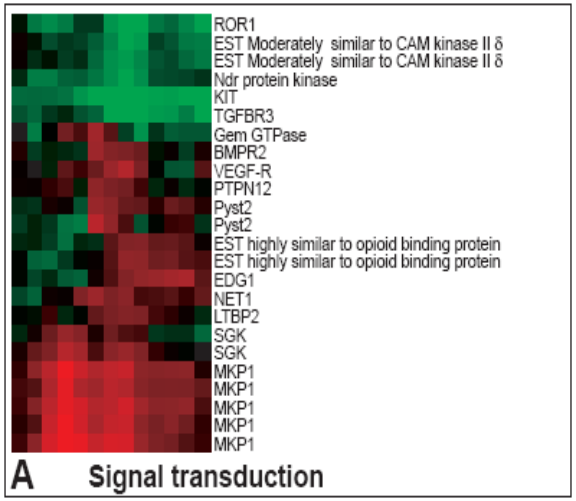
- Human fibroblasts
- Serum treatment
- Time course; 0-24 hrs
  - 12 time points
- Hierarchical clustering
  - Ordered by expression similarity

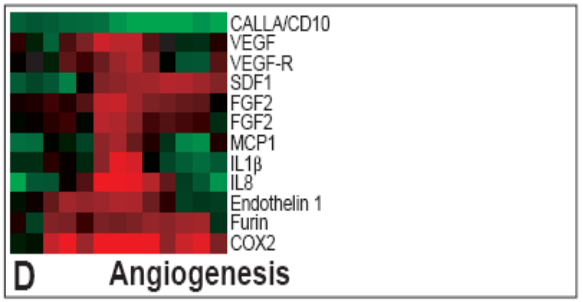


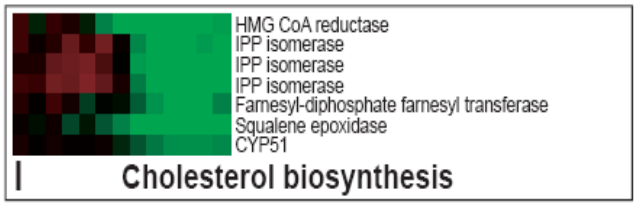
Iyer, et al
Science 283 (1999) 83-87

Time course experiment 3

- Human fibroblasts
- Sorted according to gene function (pre-GO)







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Other approaches

- A set of “related” genes
  - Simplified clustering; in the set, or not
- “Related” using any criterion:
  - Enriched in EST data
  - Cluster from gene expression
  - Sequence similarity
  - Contains a specific sequence pattern
  - Forms a complex
  - …

Uncharacterized genes 1

- Goal: Identify possible classification of a previously uncharacterized gene

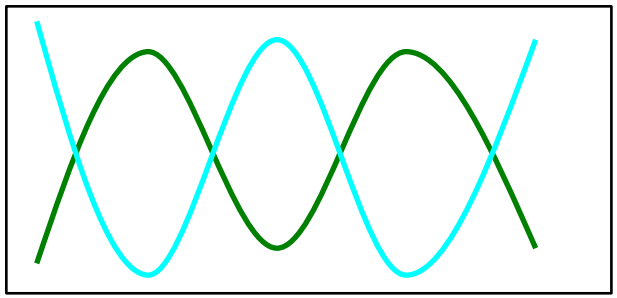
- “Guilt by association”
  - If your friend is guilty, then so are you!
  - If gene X and Y behave similarly in an experiment, then both may be involved in the same biological process

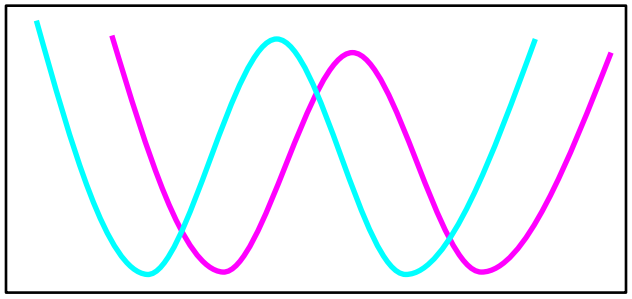
Uncharacterized genes 2

- Similar gene behavior is significant if…
  - Statistically significant
  - Some genes well-characterized, as basis
  - Well-designed experiment
- Still, may be artifact
  - Spurious correlations
    - Price of Cuban rum vs. Swedish pharmacist salary
  - Indirectly related

Uncharacterized genes 3

- But: Other types of correlation?
  - Anti-correlated
  - Phase-shifted
  - Other possibilities?
- Depends on biology





GOSt

- Data mining tool
  - Gene Ontology
  - Start from a set of genes
    - E.g. co-regulated in gene expression
    - Any other selection criterion
  - Find “enriched” GO terms
    - Give hints for uncharacterized genes
- http://www.bioinf.ebc.ee/GOST/
- Jüri Reimand, Jaak Vilo (Tartu)

Data mining 1

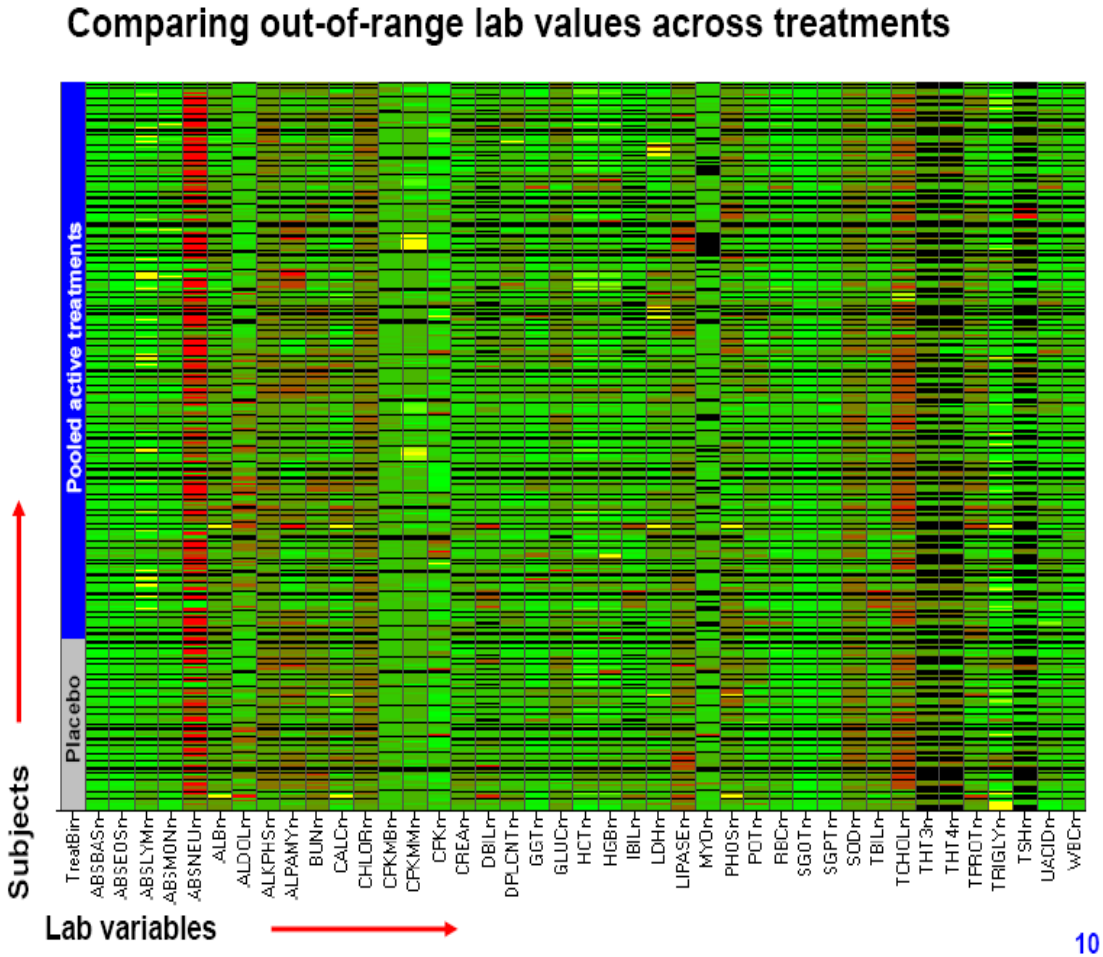
- Given large amount of data…
  - Many different dimensions (types)
  - Many data points
- How to find interesting features
  - Correlations
  - Patterns
  - Outliers

Data mining 2

- Visualization is fundamental
  - Look at the data!
  - Look again, different angle!
  - Clustering, and similar, are never enough
- Many dimensions
  - Increasingly common in clinical setting
  - Novel tools
    - Commercial: Spotfire, AVS,…
    - Open source: Mondrian,…

Data mining 3

- Patients
- Parameters
  - Clinical data
  - Biomarkers
  - Treatment
- Spotfire visualisation



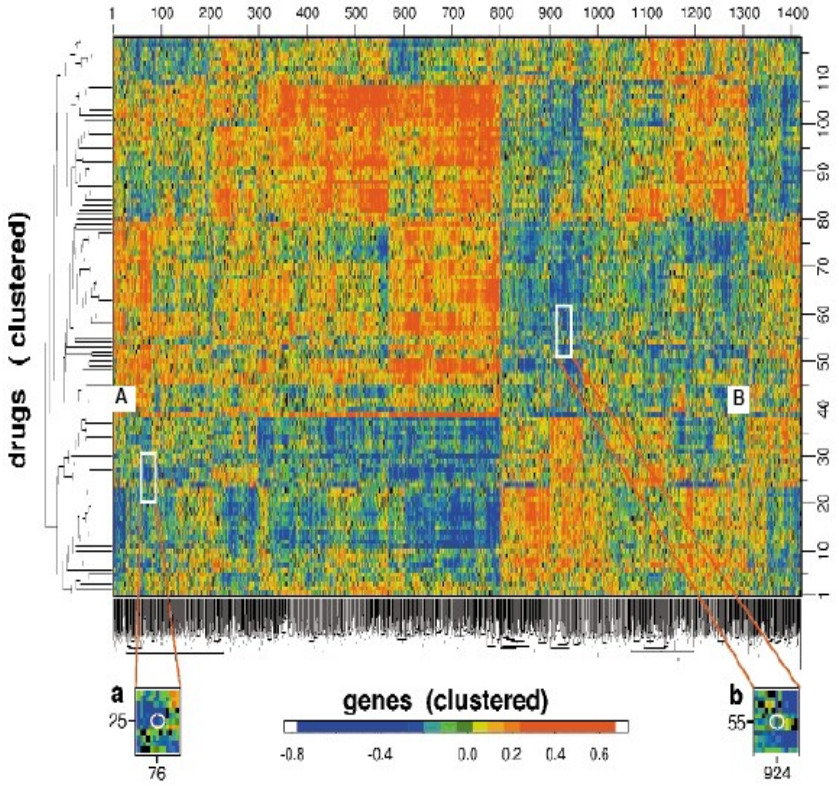
Michael Merz, Novartis
Presentation 2006
Spotfire web site

Data mining 4

- Clustering is an example of data mining
- Useful in many contexts
  - Gene expression
  - Clinical data
  - Text analysis; text mining
- Also with smallish data sets
  - Example: 20 patients, 8 parameters
    - Found 1 clear outlier using Spotfire visualization

Data mining 5

- NCI cancer drugs
  - 118 drugs
  - 60 human cancer cell lines
  - 8000 genes
- Correlation values
- Clustering



Scherf et al
Nature Genetics 24 (2000) 236-244

Gene expression data

- Databases
  - Gene Expression Omnibus at NCBI http://www.ncbi.nlm.nih.gov/geo/
  - ArrayExpress at EBI http://www.ebi.ac.uk/arrayexpress/
  - GNF SymAtlas (Novartis Research Foundation) http://symatlas.gnf.org/SymAtlas/
- Molecular Pharmacology of Cancer (NCI) http://discover.nci.nih.gov/nature2000/